PHYSIOLOGICAL CHARACTERIZATION OF A BROAD SPECTRUM REDUCTIVELY DECHLORINATING CONSORTIUM

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A wetland sediment-derived microbial consortium (WBC-2) was developed by the U.S. Geological Survey and propagated *in vitro* to large quantities by SiREM Laboratory for potential use in bioaugmentation applications. On the basis of bench-scale tests, the consortium has the capacity to completely dechlorinate 1,1,2,2-tetrachloroethene, tetrachloroethene, trichloroethene, 1,1,2-trichloroethane, *cis*- and *trans*-1,2-dichoroethene, 1,1-dichloroethene, 1,2-dichloroethane, and vinyl chloride in culture medium. Prior to field application in a bioaugmentation pilot test in a wetland, the physiological characteristics of the consortium were evaluated to improve our understanding of the fundamental microbiology of reductive dechlorinating consortia and of the potential effects of delivery methods and variable *in situ* conditions on bioaugmentation efficiency. The sensitivity of WBC-2 to pH, oxygen, and carbon tetrachloride (CT) was evaluated.

Batch microcosms were conducted under anaerobic conditions in culture medium with neutral pH and with pH adjusted from acidic (pH 4, 5, and 6) to alkaline (pH 8 and 9). The ability of the consortium to fully dechlorinate the suite of chlorinated ethanes and ethenes shown to degrade in previous studies was evaluated for each pH-adjusted treatment. The consortium was intolerant of acidic conditions of pH 5 and lower, resulting in a loss of dechlorinating ability. The consortium was tolerant of alkaline pH with no apparent loss of activity.

Reductive dechlorinating organisms commonly have high negative sensitivity to oxygen exposure over short timeframes, which complicates their field application. To evaluate oxygen sensitivity of WBC-2, an aliquot was removed from an anaerobic culture vessel and poured into smaller containers on the bench top where a series of oxygen exposures were applied to the culture by bubbling ambient air through the culture at a rate of approximately 100 milliliters per minute. Following timed exposures of 1, 5, 20, and 60 minutes, each treatment was purged with anaerobic gas (80% nitrogen: 20% carbon dioxide), sealed, and amended with 1,1,2,2-tetrachloroethane, 1,1,2-trichloroethane, and *cis*-1,2-dichloroethene, and an electron donor. Treatments were monitored for dechlorinating activity for 11 days or until complete dechlorination was observed. Dechlorinating activity was observed to be comparable to the culture with no exposure to oxygen up to an exposure time of 20 minutes. After 60 minutes of exposure, dechlorination activity was partially inhibited but eventually continued to completion during incubation under strict anaerobic conditions.

Chlorinated methanes tend to inhibit activity of a wide range of microorganisms. To test the sensitivity of WBC-2 to CT, a series of batch experiments in culture medium were conducted along with flow-through column experiments with a bioaugmented, wetland-like, organic matrix. Although toxicity effects from CT addition were observed with WBC-2 in liquid culture at a concentration of 3 milligrams per liter (mg/L), WBC-2 in the columns could maintain

degradation of CT and chloroform (CF) and of the chlorinated ethanes and ethenes at CT and CF concentrations of 10 and 20 mg/L, respectively.

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